ML00253764 at the experimental IC_{50} obtained after 72 hours. After the treatment, the cells were scraped off and recovered with their medium into blind ended tubes and counted in the Burker chamber. An aliquot containing 50,000 cells was centrifuged for 10 minutes at 800 rpm, the pellet thus obtained was lysed with an appropriate volume of lysis buffer for 30 minutes at room temperature, then again centrifuged at 200 g for 10 minutes. The supernatant was used for apoptosis analysis by ELISA assay (Cell Death detection ELISA PLUS, Roche). The ELISA apoptosis assay was performed in 96-well microplates and the absorbance reading was performed by using the Thermo labsystems Multiskan Spectrum reader (M-Medical, MI). To visualize the apoptotic process, 2×10^4 A-2058 cells were plated on slides in a 12-well plate and treated for 72 hours with the compound ML00253764 at a concentration of 10 nM. At the end of the incubation with the drug, the cells were washed with PBS, fixed and stained with TO-PRO3 iodide. The coverslips were finally mounted, and the slides viewed under a Leica TCS SP5 confocal laser scanning microscope. Apoptosis was confirmed by the characteristic, intensely fluorescent "shrunken" nuclei.

EXAMPLE 5: MC4R HISTOCHEMISTRY IN MELANOMA CELLS

[0060] In order to study the MC4R protein in WM-266-4 and A-2058 cell lines, 2×10^4 cells were plated on a slide in a 12-well plate. After 72 hours, the cells were fixed in a PBS (pH 7.4) and 4% paraformaldehyde solution for 1 hour at room temperature. The fixed cells were then incubated in a blocking solution consisting of 15% goat serum and 0.2% Triton X-100 in PBS for 30 minutes. The cells were then incubated overnight at 4° C. with the anti-MC4R antibody at a concentration of 20 μg/ml in PBS. The cells were washed with PBS and incubated with the biotinylated anti-rabbit IgG antibody and subsequently with the peroxidase-conjugated avidin-biotin complex. Peroxidase was detected by using diaminobenzidine and hydrogen peroxide. The negative control was performed by omitting the primary antibody. For the immunofluorescence assay, the slides were incubated for 90 minutes with a green-fluorescent goat anti-rabbit secondary antibody diluted 1:250 in PBS. Nuclear staining was performed by incubating the cells with TO-PRO3 diluted 1:1,000 in PBS (pH 7.4) for 15 min. Negative controls were obtained with cells incubated without the primary antibody.

EXAMPLE 6: MC4R WESTERN BLOT ANALYSIS

[0061] The lysis buffer (RIPA containing a protease inhibitor mix) was added to the melanoma, gastrointestinal carcinoma and thyroid carcinoma cells, as well as to normal human endothelial cells (HUVEC). After an incubation period of 15 minutes on ice, the cells were sonicated and then centrifuged at 14,000×g for 15 minutes at 4° C. The supernatants were collected, and the protein content was determined. Proteins were denatured, separated by electrophoresis and then blotted onto membranes by using a blotting buffer. The membranes were then blocked, washed and incubated with the primary antibody to MC4R (1:500 dilution) overnight at 4° C. After 24 hours, the membranes were incubated with a specific peroxidase-conjugated secondary antibody (Cell Signaling Technology; Danvers, Mass., USA) for 1 hour at room temperature. To verify an equal loading of the wells, β-tubulin expression was analyzed using an anti-β-tubulin antibody. After washing, the membranes were analyzed by chemiluminescence. The bands related to MC4R and β-tubulin (37 and 55 kDa, respectively) were quantified by densitometric scanning through imaging (Bio-Profil, Celbio, Italy). The level of all bands was expressed as the relative integrated intensity normalized to β-tubulin (=100). The data are presented as mean values±S.E.M. for 8-10 repetitions for each cell line.

EXAMPLE 7: STATISTICAL ANALYSIS

[0062] The results of the data obtained herein (mean of the values±S.E.M.) were subjected to statistical analysis by ANOVA followed by Newman-Keuls test or by Student's T-test, using the GraphPad Prism software (version 5.0; GraphPad Prism Software Inc., San Diego, Calif., USA). The data significance level was set for P values <0.05.

EXAMPLE 8: MC4R RECEPTOR EXPRESSION IN MELANOMA, GASTROINTESTINAL TUMOR AND THYROID CARCINOMA CELL LINES

[0063] Immunohistochemical analysis, carried out by the present inventors as shown in Example 5, revealed for the first time that MC4R receptor is significantly expressed in both human melanoma cell lines A-2058 (FIG. **1**A) and WM-266-4 (FIG. **2**A) when compared to the negative controls in the absence of the primary antibody (FIGS. **1**B and **2**B). It should be noted that no significant differences were observed between A-2058 and WM-266-4 cells with regard to immunohistochemistry positivity (FIGS. **1** and **2**). Furthermore, the immunofluorescence of proliferating cells clearly revealed the presence of the MC4R protein on cell membranes of A-2058 melanoma cells (FIG. **3**A), when compared to the negative controls in the absence of the anti-MC4R primary antibody (FIG. **3**B).

[0064] The results of the immunohistochemical analysis were then confirmed by Western blot analysis, which revealed the presence of a high concentration of MC4R protein in human melanoma cells compared to normal HUVEC cells (FIG. **4**, top). MC4R levels in human melanoma cells were significantly higher than in endothelial cells in the computerized image analysis of eight to ten repetitions for each cell line (FIG. **4**, graph below).

[0065] By Western blot analysis the present inventors also demonstrated for the first time a significant presence and expression of the MC4R receptor in the Caco-2 (FIG. **5**A) and HT-29 (FIG. **5**B) gastrointestinal tumor cell lines, as well as in the 8305C thyroid carcinoma cell line (FIG. **5**C). A higher concentration of MC4R protein compared to normal HUVEC cells was observed in all cell lines examined (FIGS. **5**A, **5**B and **5**C, top), and MC4R levels in Caco-2, HT-29 and 830C cells were significantly higher than in endothelial cells in the computerized image analysis of eight to ten repetitions (FIGS. **5**A, **5**B and **5**C, graphs below).

EXAMPLE 9: ANTIPROLIFERATIVE EFFECT OF THE MC4R RECEPTOR ANTAGONIST ON MELANOMA, GASTROINTESTINAL TUMOR AND THYROID CARCINOMA CELLS

[0066] The antiproliferative activity of the MC4R receptor antagonist for use according to the invention was first studied in BRAF-mutant melanoma cell lines. As shown in